REMARKS

Claims 1-64 were pending in the subject application. By the present Amendment, applicants have canceled non-elected claims 1-19, 21, 22, 24, 26-30, 32, 34-48, 58, and 60-63, and additionally claims 23, 25, 31, 33, 52, 53, and 64, without disclaimer or prejudice to applicants' right to pursue the subject matter of these claims in this or another application. Applicants have also amended claims 20, 49, 50, 51, 54-57, and 59. The amendments to claims 50 and 54-56 involve merely formatting changes and do not raise any issue of new matter. Claim 49 was amended at the request of the Examiner to add a step to the recited method. The other amendments are fully supported in the specification as follows: Claim 20: page 9, lines 8-9, 14-15 and 20-21, and line 26 to page 10, line 5; Claim 51: page 14, lines 22-26; Claims 57 and 59: page 7, lines 11-17; page 14, lines 22-23. Thus, applicants maintain that these amendments do not present new matter. Accordingly, applicants respectfully request that the Examiner enter this Amendment. Upon entry of this Amendment, claims 20, 49-51, 54-57, and 59, as amended, will be pending and under examination.

Rejections under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 49, 50, 54 and 55 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

The Examiner stated that claims 54 and 55 had insufficient antecedent basis. Claims 54 and 55 have been amended, thus rendering this ground of rejection moot.

The Examiner also stated that claim 49 is incomplete for omitting essential steps. In response, applicants note that the claimed method is not limited to expressing an ion channel in a heart. Claim 49 has been amended to include a first step for preparing a composition. Accordingly, applicants respectfully request withdrawal of this ground of rejection.

Rejection of claims 49-57 and 59 under 35 U.S.C. §112, First Paragraph (Enablement)

The Examiner rejected claims 49-57 and 59 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement.

Applicants respectfully disagree. Applicants note that the specification teaches the stable transfection of stem cells with a chosen pacemaker gene (see page 21, lines 6-18). Suitable pacemaker channels are identified as HCN1, HCN2, HCN4 and mutated HCN alpha subunits and the MiRP1 beta subunit (page 9, line 11 to page 10, line 8; page 18, lines 23-25; page 19, lines 9-19 and 21-23). Applicants note that the transfection of cells involves routine methodology well known in the art. This routine methodology need not be described in detail in the specification since, to satisfy the enablement requirement, "a patent need not teach, and preferably omits, what is well known in the art." *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986). See also *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991) ("The specification need not disclose what is well known in the art"). In addition, the specification identifies vectors that may be used to constitutively express HCN genes in transfected cells. See page 21, lines 11-16.

Applicants note that they recently demonstrated the stability of HCN2 expression in stem cells transfected as described in the specification. As reported in Plotnikov et al. (2005) Circulation 112: II-126 (copy attached hereto as **Exhibit A**), stem cells transfected with HCN2 and introduced into canine hearts *in situ* induced pacemaker function which was stable for at least 6 weeks, i.e., over the course of a 42-day-long study, all animals showed stable pacemaker activity for the duration of the study.

The specification also discloses that solutes, including inorganic ions, in stem cells can be transferred via diffusion through gap junctions to target cells contacted with the stem cells (page 32, lines 16-24). The specification further provides experimental data demonstrating the transfer of the Lucifer Yellow dye from a stem cell to a HeLa cell expressing the connexin, Cx43 (page 6, lines 8-13; page 33, lines 4-7; Figure 2); coupling and ionic and dye transfer between a stem cell and a canine cardiomyocyte (page 6, lines 15-21; Figure 3); and coupling and ionic and dye transfer between a stem cell and a HeLa-$Cx43^+$ cell (page 6, lines 23-26; Figure 4). The specification teaches that stem cells form gap junction channels with other cells expressing one or more of the following connexins: Cx43, Cx45, Cx40, Cx32 and Cx26 (page 33, lines 10-12). The specification also teaches that transfer of the Lucifer Yellow to the HeLa-$Cx43^+$ cell likely occurs by diffusion through gap junctions (page 6, lines 12-13).

Thus, applicants disagree with the Examiner's assertion that in the absence of any specific staining for gap junctions, it is "difficult to extrapolate" that engrafted cells form gap junction with cardiac cells. Applicants maintain that the demonstrated transfer of Lucifer Yellow from stem cells to cardiomyocytes and HeLa-Cx43^{+} cells, respectively, as disclosed in the specification, is *prima facie* evidence of robust gap junction-mediated coupling. Moreover, applicants have confirmed the formation of gap junctions between MSCs and myocytes using both immunostaining and western blotting techniques. See Valiunas et al. (2004) J. Physiol. 555: 617-626 (copy attached hereto as **Exhibit B**; see Abstract, Fig. 1 and accompanying text), and Potapova et al. (2004) Circ. Res. 94: 952-959 (copy attached hereto as **Exhibit C**; see Abstract, Fig. 8 and accompanying text). Potapova et al. (2004) (**Exhibit C**) specifically demonstrate that engrafted stem cells formed gap junctions with native cardiomyocytes. In addition, Valiunas et al. (2004) (**Exhibit B**) also demonstrate that electrical current flowed through the gap junctions. Thus, applicants maintain that the ability of engrafted cells to form gap junction with cardiac cells is not in dispute. Moreover, applicants reiterate that the specification as filed provides strong evidence for the formation of these gap junctions.

The specification also teaches and provides experimental data confirming that the HCN2 gene incorporated into stem cells generates a pacemaker current (page 7, lines 11-17; Figure 8). It further teaches, supported by experimental data, how to use such stem cells to induce a pacemaker current in a subject's heart by delivering human MSCs incorporated with the HCN2 gene *in situ* into a canine heart (page 7, lines 28 to page 8, line 18; Figure 10).

Claim 51, as amended, provides a method of treating a cardiac rhythm disorder in a subject, which comprises contacting a cell of the heart with a MSC incorporated with a nucleic acid encoding a HCN2 channel in an amount effective to increase pacemaker current expression of the cell. As noted above, the specification provides an experimental demonstration of the use of MSCs incorporated with the HCN2 gene to induce a pacemaker current in a canine heart. The specification also discloses a method of treating a cardiac condition in a subject which comprises contacting a cell of the heart of the subject with a composition comprising stem cells that have been incorporated with a compound in an amount sufficient to create ion channels and increase the current expression of the cell (page 20, lines 14-31). Thus, applicants maintain that the specification provides a fully enabling

disclosure in support of claim 51, as amended.

The specification discloses that stem cells expressing the HCN2 gene to induce a pacemaker current can be used to treat cardiac rhythm disorders including block, complete atrioventricular block, incomplete atrioventricular block, and sinus node dysfunction (page 20, lines 22-31). Applicants maintain that determining the level of ion channel gene expression sufficient to create ion channels and increase the current expression of a cell requires only routine experimentation, for example, in evaluating the use of different promoters to drive gene expression, and measuring the resulting level of current expression in the cell. Moreover, applicants note that both Plotnikov et al. (2005) (**Exhibit A**) and Potapova et al. (2004) (**Exhibit C**) demonstrate that genetically modified hMSCs functionally expressed HCN2 at a level sufficient to create ion channels that induce effective pacemaker activity in canine hearts *in situ*.

In view of the above-identified disclosures in the specification detailing (1) how to make the invention, and (2) how to use it, applicants maintain that the invention now claimed in the subject application is fully enabled by the specification as filed. Applicants respectfully direct the Examiner's attention to M.P.E.P §2164.01(b) which states that as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. §112 is satisfied.

The Examiner conceded that the specification shows the role and importance of implantation of stem cells incorporated with the HCN2 gene in canine heart for pacemaker activity, but stated that the disclosures do not demonstrate the information required by the artisan to reasonably predict the optimal number stem cells that are required for pacemaker activity for the treatment of any cardiac condition as claimed. The Examiner further stated that the specification does not provide any specific guidance of any correlation between the numbers of cells required for successful pacemaker activity for a sustained period. The Examiner also stated that a skilled artisan would have to perform undue experimentation to ensure that HCN2 is stably expressed for a long duration in order to express the ion channel gene for sustained pacemaker activity.

Applicants respectfully disagree and submit that the claims as amended are fully enabled. As noted above, Plotnikov et al. (**Exhibit A**) demonstrated at least 6 weeks of stable pacemaker function induced with stem cells transfected with HCN2 and introduced into canine hearts *in situ*. This experiment involved injection of 1,000,000 stem cells..

Applicants note that the instant grounds of rejection appear to be based on the absence of an optimized protocol in the specification for performing the claimed methods. However, it is respectfully submitted that provision of an optimized protocol is not required to satisfy the enablement requirement. *In re Wands* makes clear that the relevant factor in assessing enablement is not so much the amount of experimentation required to practice an invention, but whether the experimentation required is routine:

> Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. ... The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, ... (emphasis added) *In re Wands*, 8 U.S.P.Q.2d 1400, 1404.

Applicants maintain that, based on the disclosures in the specification coupled with the routine methodology known in the art, a skilled practitioner could use the invention employing only routine experimentation. Applicants respectfully request, therefore, that the instant grounds of rejection be withdrawn.

Rejection of claims 51-52, 57 and 59 under 35 U.S.C. §112, First Paragraph (Enablement)

The Examiner stated that claims 51-52, 57 and 59 read on a method for treating any heart condition in a subject which comprises an *in vivo* method of delivering any stem cell incorporated with any compound. The Examiner contended that the specification as filed does not provide sufficient guidance or factual evidence for one skilled in the art to practice the claimed method.

Applicants note that claims 52 and 53 have been canceled, and claim 51 has been amended to recite a method of treating a cardiac rhythm disorder. Applicants respectfully submit that this amendment renders the present ground of rejection moot.

The Examiner also cited various journal articles relating to certain safety issues. In response, applicants maintain that the study by Zhang, (2002) Circulation 106: 1294-9, is completely irrelevant for the following reasons. First, Zhang evaluated the electrophysiological properties of cardiomyocytes, differentiated *in vitro* from embryonic stem cells and embryonal carcinoma cells, to determine the extent to which they might show arrhythmic proclivity. In contrast, the subject invention encompasses the use of undifferentiated, adult MSCs, incorporated with a HCN2 gene, to induce a pacemaker current. Second, the embryonic stem cells used by Zhang are pluripotent and have innate pacemaking function. In contrast, hMSCs as used in the subject invention are multipotent, have no innate pacemaking function, and exhibited no evidence of differentiation or generating arrhythmias even after six weeks of monitoring. See Plotnikov et al. (2005) (**Exhibit A**).

Moreover, applicants maintain that the safety issues raised by the Examiner fall within the province of the Food and Drug Administration (FDA) and not the Patent Office. In this regard, applicants respectfully direct the Examiner's attention to *In Re Brana* (51 F.3d 1560, 1567, 34 U.S.P.Q.2d 1436, 1442, Fed. Cir. 1995):

> The Commissioner counters that such in vivo tests in animals are only preclinical tests to determine whether a compound is suitable for processing in the second stage of testing, by which he apparently means in vivo testing in humans, and therefore not reasonably predictive of the success of the claimed compounds for treating cancer in humans. The Commissioner, as did the Board, confuses the requirements under the law for obtaining a patent with the requirements for obtaining government approval to market a particular drug for human consumption. *See Scott v. Finney*, 34 F.3d 1058, 1063, 32 U.S.P.Q.2d 1115, 1120 (Fed. Cir. 1994) ("Testing for the full safety and effectiveness of a prosthetic device is more properly left to the Food and Drug Administration (FDA). Title 35 does not demand that such human testing occur within the confines of Patent and Trademark Office (PTO) proceedings.") (emphasis added).

Applicants therefore maintain that safety concerns regarding, for example, the potential for cell transplantation therapy to generate unanticipated arrhythmias and the need to solve safety and efficacy problems before clinical evaluation in human beings, fall outside the requirements for patentability. Accordingly, applicants maintain that the instant ground of rejection is without merit and respectfully request its withdrawal.

The Examiner stated that claims 51, 57 and 59 refer to current expression in a cell of the heart, which encompasses several types of current in the heart (citing, e.g., potassium and sodium currents), and noted that HCN2 is only associated with increasing expression of the pacemaker current (citing Proenza et al. (2002) J. Biol. Chem. 277: 5101-9). The Examiner asserted that the claimed methods are thus not enabled for inducing any other current in the heart. Applicants respectfully submit that present claim amendments ("effective to increase pacemaker current") render this ground of rejection moot.

The Examiner cited a study by Léobon et al., (2003) Proc. Natl. Acad. Sci. U.S.A. 100(13): 7808-11 (henceforth "Léobon"), showing that grafted myoblasts differentiate into peculiar hyperexcitable myotubes with a contractile activity fully independent of neighboring cardiomyocytes. The Examiner also stated that this study raises the issue of unpredictability in the art of electro-mechanical coupling of grafted cells.

Applicants respectfully disagree. Applicants note that, as conceded by the Examiner, the procedures studied by Léobon, involving the transplantation of myoblasts into infarcted myocardium, differ from the methods of the claimed invention which involve the use of stem cells to induce pacemaker currents in target cells. Thus, Léobon's results are largely irrelevant to enablement of the instant claims. To the extent these results raise questions about unpredictability in the art of electro-mechanical coupling of grafted cells, potential altered biophysical and trans-differentiation of stem cells during prolonged expression of transgenes in the heart, applicants maintain that this too is irrelevant to the claimed invention. For instance, whereas the stem cells themselves do no beat, the cardiac cells they couple to and the remainder of the heart are both induced to beat by the pacemaker current transmitted by the stem cells. In fact, the action of the stem cells is to transmit current via the gap junctions, which results in the consistent beating of the cardiac myocytes. Applicants note that electromechanical coupling is a function of the heart cells only and not the stem cells, which <u>couple electrically, but not electromechanically,</u> to the heart cells.

Moreover, applicants respectfully remind the Examiner that any questions regarding possible adverse effects of prolonged expression of transgenes in the heart on pacemaker activity fall within the ambit of the Food and Drug Administration (FDA) and <u>not</u> the Patent Office. Accordingly, applicants respectfully request that the Examiner reconsider and

withdraw this ground of rejection.

The Examiner has alleged that the specification and prior art do not teach a method of administering genetically modified stem cells in humans, and that the specification lacks specific guidance and direction and/or working examples. In response, applicants note that methods for transfecting cells with foreign genes, such as by viral infection and electroporation, are well known in the art. It was therefore clear that, as it with any other type of cell, hMSCs could be readily transfected.

Further, applicants note that the specification describes the *in situ* administration of genetically modified stem cells to canine hearts, so as to thereby induce an effective pacemaker current in the heart. See page 7, lines 28 to page 8, line 18; Figure 10. Applicants maintain that this canine model is an appropriate model for, and a working example of, the application of the claimed method to the treatment of humans. See M.P.E.P. §2164.02:

> The issue of "correlation" is related to the issue of the presence or absence of working examples. "Correlation" as used herein refers to the relationship between *in vitro* or *in vivo* animal model assays and a disclosed or a claimed method of use. An *in vitro* or *in vivo* animal model example in the specification, in effect, constitutes a "working example" if that example "correlates" with a disclosed or claimed method invention. ... Since the initial burden is on the examiner to give reasons for the lack of enablement, the examiner must also give reasons for a conclusion of lack of correlation for an *in vitro* or *in vivo* animal model example.

Based on the foregoing remarks, applicants maintain that the specification as filed enables one skilled in the art to make and use the invention recited in the presently amended claims. Accordingly, applicants respectfully request withdrawal of this ground of rejection.

Rejections under 35 U.S.C. §112, First Paragraph (Written Description)

The Examiner rejected claims 20, 23, 25, 31, 33, 49-57, 59 and 64 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement.

Applicants respectfully disagree and submit that the present claim amendments render this ground of rejection moot. Claims 20, 49-57, and 59, as amended, specifically encompass a nucleic acid, which encodes a HCN2 ion channel, treating a cardiac rhythm disorder, to

increase a pacemaker current, and using a mesenchymal stem cell. Accordingly, applicants respectfully request that the Examiner withdraw this ground of rejection.

Rejection of claim 64 under 35 U.S.C. §102(b) and §102(e)

The Examiner rejected claim 64 under 35 U.S.C. §102(b) as allegedly anticipated by Gerson et al., U.S. Patent No. 5,591,625, issued January 7, 1997, and §102(e) as allegedly anticipated by Pittenger et al., U.S. Patent No. 6,387,369, issued May 14, 2002; effective filing date March 27, 2000.

Claim 64 has been canceled, thus rendering this ground of rejection moot. Accordingly, applicants request withdrawal of this ground of rejection.

Rejection of claims 20, 23 and 25 under 35 U.S.C. §102(e)

The Examiner also rejected claims 20, 23, and 25 under 35 U.S.C. §102(e) as being anticipated by Marban et al., U.S. Patent Publication No. US 2004/0254134, published February 16, 2004; effective filing date February 29, 2002. The Examiner stated that claims 20, 23, and 25 are drawn to a composition for ion channel transfer, which comprises stem cells incorporated with a compound in an amount sufficient to create an ion channel. The Examiner noted that subsequent claims limit the compound to HCN2.

Applicants respectfully disagree. Applicants note that claims 23 and 25 have been canceled hereinabove. However, the subject matter of these canceled claims has been incorporated into claim 20, as amended. Accordingly, applicants respond to the instant rejection as it applies to claim 20, as amended.

Applicants note that claim 20, as amended, is directed to a composition comprising a MSC incorporated with a nucleic acid encoding a HCN2 ion channel. Applicants assert that Marban does not disclose a mesenchymal stem cell, far less a mesenchymal stem cell incorporated with a nucleic acid encoding a HCN2 ion channel.

Since a finding of anticipation requires that a prior art reference teach each and every element of the rejected claims, and Marban does not disclose a mesenchymal stem cell incorporated with a nucleic acid encoding a HCN2 ion channel, applicants maintain that the

instant claims are not anticipated by Marban. The Examiner is therefore requested to reconsider and withdraw the instant ground of rejection.

Rejections under 35 U.S.C. §103(a)

The Examiner rejected claims 20, 23, 25, 27, 31, 33, and 64 under 35 U.S.C. §103(a) as being unpatentable over Rosen et al., U.S. Patent No. 6,849,611; effective filing date June 6, 2001 (henceforth "Rosen"), in view of Heubach et al. (2002) Circulation 106(19) Suppl: 11-68 (henceforth "Heubach").

In response, applicants respectfully traverse. Applicants note that claims 23, 25, 27, 31, 33, and 64 have been canceled, thus rendering their rejection moot. However, the subject matter of canceled claims 23 and 25 has been incorporated into claim 20, as amended. Accordingly, applicants respond to the instant rejection as it applies to claim 20, as amended.

Applicants maintain that the Examiner has failed to establish a *prima facie* case of obviousness of claim 20 for the following reasons. First, applicants note that the subject application claims priority to U.S. Provisional Application No. 60/440,265, filed January 15, 2003, and is entitled to an effective filing date of January 15, 2003. Second, applicants note that the Rosen reference cited by the Examiner was published by applicants on December 12, 2002, i.e., less than one year prior to the effective filing date of the subject application. Accordingly, Rosen is not prior art under 35 U.S.C. §102(b) with regard to the subject application.

Since the Rosen reference is not published "by another," it is also not prior art under 35 U.S.C. §102(a), (e), (f) and (g). 35 U.S.C. §102(c) and (d) do not apply. Accordingly, Rosen is not prior art and cannot be the basis under 35 U.S.C. §103(a) for an obviousness rejection. Applicants therefore respectfully request that the Examiner withdraw this ground of rejection.

Conclusion

In view of the remarks made hereinabove, applicants respectfully request that the Examiner reconsider and withdraw the rejections set forth in the December 7, 2005 Office

Action, and earnestly solicit allowance of all claims now pending in the subject application.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone her at the number provided below. No fee is deemed necessary in connection with the filing of this Amendment. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 11-0600.

Respectfully submitted,
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